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Three-dimensional Cytoarchitecture of Normal and Atherosclerotic Intima of Human Aorta

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The three-dimensional cytoarchitecture of normal and atherosclerotic intima of human aorta was studied by light microscopy of consecutive* en face *preparations (Hautchen preparations) and by scanning electron microscopy. In un!ffected intima, a three-dimensional network consisting of cells of variable shape and probably origin was demonstrated. Cellular shape changed from predominantly stellate in the luminal regions of the elastic-hyperplastic layer to elongated spindlelike cells in the musculoelastic layer of the intima. In the surface layers of the fatty streak, cellular contacts were severed, and lipid droplets were often seen between cellular processes. Along with stellate and elongated cells, the fatty streak also had a number of round monocytelike cells. Lipid inclusions were usually detected in stellate and ovoid cells. The integrity of the cellular network was preserved at the marginal zone of the atherosclerotic plaque, while at the slopes and in the central part of the plaque, cells practically lost all contact with each other. Giant stellate cells embedded in crude fibrillar connective tissue matrix were often found there. Disintegration of the cellular network during atherosclerosis is suggested to play an important role in the development of various lesions. (Am J Pathol 1991, 138:569–580)

Cellular composition of human aorta has been the subject of intensive study by light[1–4] and electron microscopy[5–7] for more than a hundred years. With the advance of different cell biology methods, current attention is focused primarily on the examination of morphologic features of the subendothelial intimal cells in atherosclerosis.[8–11] Changes occurring in the cellular composition of the vessel wall due to atherosclerosis were shown to be intimately related to the main biochemical manifestations of atherosclerosis, eg, lipidosis and fibrosis.[7] These data indicate that the cells of subendothelial intima play an important role in atherogenesis. Investigations were mostly concerned, however, with the identification and characterization of cells of different types. Therefore little is yet known about the cellular network of the vessel wall as a complete system. The present study provides a detailed analysis of the three-dimensional cytoarchitecture of the subendothelial normal and atherosclerotic human aortic intima as visualized by light and scanning electron microscopy on *en face* tissue preparations.

Materials and Methods

Twelve aortas were taken 1.5 to 3 hours after death from male subjects aged 30 to 60 years, the majority of whom had died suddenly of trauma. The aortas were opened longitudinally under sterile conditions, and the adventitia was carefully dissected.[12] The remaining tissue was washed in phosphate-buffered saline (PBS), pH 7.4. The endothelium was removed by immersion and constant agitation in a solution of 0.1% dispase for 1 hour at 37°C. As demonstrated previously,[13] dispase eliminates only endothelial cells from the vessel lumen, and leaves the subendothelial layer intact. After incubation, the tissue was rinsed in PBS and fixed in a solution of 4% formaldehyde and 1% glutaraldehyde in PBS for 24 hours at 4°C. The structure of aortic intima before and after dispase treatment is shown in Figure 1A and B. It can be seen that the cells under the endothelium are exposed. The surface of the subendothelial cells is masked, however, by a network of connective tissue fibrils.

Light-microscopic Study of Intimal En Face Preparations

Intimal *en face* preparations (Hautchen preparations) were made essentially as described by Schwartz and

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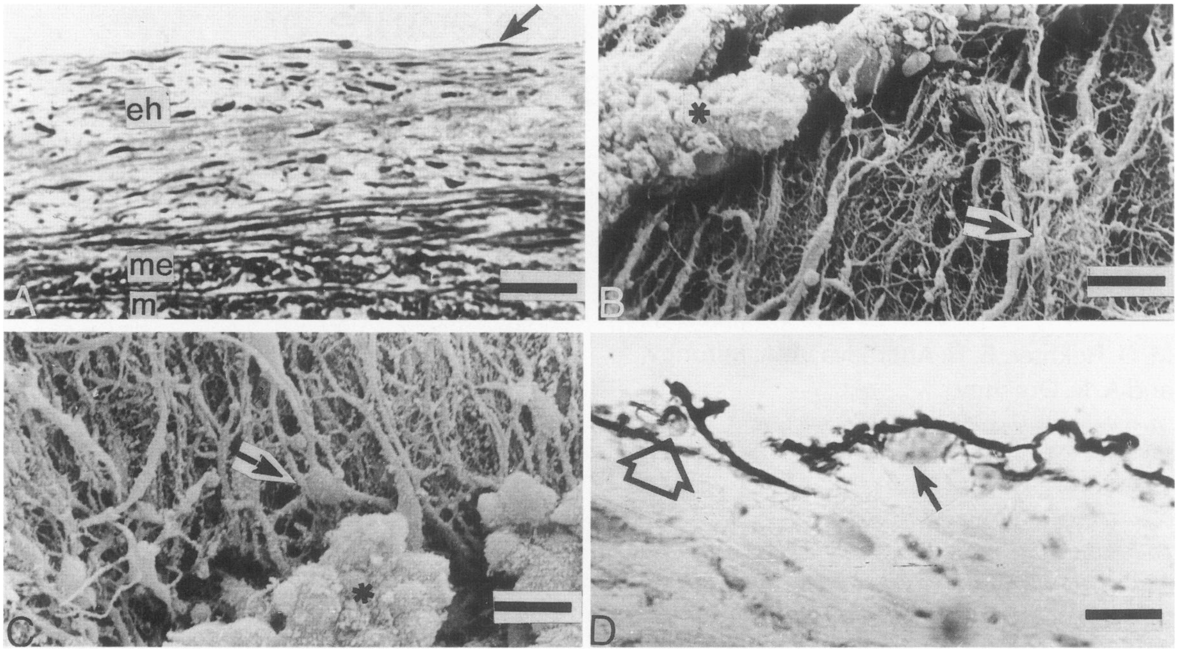

Figure 1. *Structure of grossly normal human aortic intima after treatment with dispase and maceration in HCl-collagenase solution.* **A:** *Semithin cross section of untreated aorta. M, media; ME, musculoelastic intimal layer; EH, elastic-hyperplastic intimal layer. Endothelial cells are indicated by the arrow. Methylene blue (bar, 83.3 μ; ×120).* **B:** *The inner surface of the aorta after dispase treatment. Subendothelial cells (indicated by the arrow) are masked by connective tissue network. Asterisk indicates remaining endothelial cells. SEM (bar, 12.5 μ; ×800).* **C:** *the inner surface of the aorta after dispase treatment and hydrolysis with HCl-collagenase. Subendothelial stellate cells with elongated processes (indicated by the arrow) free from obscuring matrix components can be seen. Asterisk indicates remaining endothelial cells. SEM (bar, 14.3 μ; ×700).* **D:** *Semithin cross section of the re-embedded (after SEM examination) sample treated with dispase, HCl, and collagenase. The luminal aortic surface has a spiky appearance due to the removal of extracellular matrix components between cells. Arrow indicates the body of subendothelial cell, arrowhead indicates cellular process. The dark band coating the luminal surface means a layer of sputtered metal. Methylene blue (bar, 12.5 μ; ×800).*

Benditt,[14] who developed the method for the study of vascular endothelium. Vessels treated with dispase and fixed as mentioned previously were dissected under a dissecting microscope to single out unaffected intimal regions, fatty streaks, and atherosclerotic plaques[12] not larger than 1 cm^2. Using fine forceps, most of the media was carefully removed and discarded. The remaining tissue specimens were then conventionally dehydrated in rising ethanol series. From the last, 100% alchocol solution, the pieces were transferred to celloidin (VEB Laborchemie, Apolda, FRG) (20% celloidin dissolved in a mixture of ethanol-ether, 1:1) for several seconds, and then placed intimal surface down onto glass slides previously covered with celloidin. The preparations were left under press for about 15 to 20 minutes, and were then put into 30% ethanol for 30 seconds to allow swelling of the celloidin. Cautiously, the tissue specimens were finally taken off the glass slides, with the adhering surface layer of the intima remaining on the slide. The rest of the tissue piece was replaced in 100% alcohol and successive *en face* preparations were obtained several times. Usually two to three *en face* intimal preparations from an unaffected region and up to four to six preparations from fatty streaks and atherosclerotic plaques could be collected. The thickness of the *en face* preparations (about 25 to 40 μ) was monitored on cross sections of pieces from which they were made. The celloidin *en face* preparations were stained with hematoxylin and mounted in Entellan (Merck, Darmstadt, FRG). We omitted the removal of celloidin and fixation on gelatin base described in the original paper[14] from our protocol, as celloidin had no effect on the quality of staining, and the total time spent on specimen preparation was reduced from 2 to 3 days to about 1 hour. To verify data obtained on *en face* preparations, horizontal sections of paraffin-embedded material were used. Paraffin-embedded sections were prepared as described elsewhere and stained with hematoxylin. Oil red O was used to reveal lipids in *en face* preparations and frozen tissue sections. The preparations were examined under an Opton III (Karl Zeiss, FRG) phase-contrast microscope.

Scanning Electron-microscopic Study of Intimal Preparations

The cellular network of the aortic intima was examined by scanning electron microscopy (SEM). Acid hydrolisis of fixed tissue specimens followed by collagenase treatment was used to remove obscuring extracellular matrix components.[15] After determining optimal conditions (time of acid and collagenase dispersion), the tissue samples were processed as follows. Specimens of grossly normal and atherosclerotic tissue, denuded of endothelium, were fixed, washed in PBS, and immersed in 8 N HCl for 20 minutes at 60°C. Then the samples were extensively washed in several changes of distilled water and incubated in a collagenase solution, 1 mg/ml (crab collagenase was obtained from Paralithodes camtshatica, 100 UI/mg, Pacific Institute of Biological and Organic Chemistry, Vladivostock, USSR)[16] for 3 hours at 37°C. After prolonged rinsing in distilled water, the tissue pieces were prepared for SEM: postfixed in 1% osmium tetroxide for 1 hour, followed by washing in distilled water, stained with 1% tannic acid for 30 minutes, dehydrated in graded ethanols starting from 30°, two changes of acetone for 5 minutes, and critical-point–dried in an HCP-2 (Hitachi, Tokyo, Japan), mounted on aluminium supports. The specimens were sputter coated with gold in a JFC-1100 (Hitachi) apparatus and examined in a Hitachi S-570 scanning electron microscope under an accelerating voltage of 20 kV. The surface of subendothelial cells was totally cleared from obscuring connective tissue fibrils by the HCL-collagenase procedure described above (Figure 1C, D).

To study cells located in deeper areas of the aortic wall, the surface layers were carefully stripped away using microdissecting forceps, and the emerging surface was subjected to treatment with hot HCl and collagenase, essentially in the same manner as described for the subendothelial layer.

Precise identification of SEM-visualized structures and localization of layers in the total intima was conducted on semithin cross sections. For this purpose, the specimens were detached from their supports and incubated in absolute acetone for 10 minutes, embedded in a mixture of Epon-812 (E. Fullam, Inc., Latham, NY) and Araldite (Fluka, Buchs, Switzerland). Semithin sections were cut from each block on an LKB-III ultramicrotome (LKB, Stockholm, Sweden) and stained with methylene blue. Some blocks were chosen for further preparation of ultrathin sections, which were counter-stained with uranyl acetate and lead citrate according to conventional methods and examined under an electron microscope EMV-100AK (Electron, Sumie, USSR).

Results

Human aortic intima is separated from the media by an internal elastic membrane. The secondary elastic membrane, the inner limiting membrane, divides the intima into two layers, the elastic-hyperplastic (adjacent to the vessel lumen) and musculoelastic (adjacent to the media) (Figure 1A).

The three-dimensional cellular network of the intima was examined on consecutively prepared intimal *en face* preparations and by SEM. *En face* preparations suitable for investigation could be obtained only from the uppermost layer of the intima. Deeper areas of the musculoelastic layer contained a well-developed elastic fibrillar network, which interfered with the preparation of *en face* samples of muscular layer. Therefore, we studied deeper intimal layers predominantly by SEM, while the cellular network of surface intimal layers was examined by light microscopy in combination with SEM.

Normal Intima

The juxtaluminal intimal layer of grossly normal intima was separated into two to three *en face* samples. In the surface layer (adjacent to the endothelium), light microscopy showed a network of cells, most of which were stellate shaped (Figure 2A). The subendothelial cells were unevenly distributed throughout the tissue in a clusterlike manner with areas densely populated by cells interchanging with areas having few cells (Figure 2B). We also found clustering of intimal cells by more conventional horizontal sectioning technique on paraffin-embedded material (Figure 2C). Cells in the network had similar dimensions. The layer of stellate cells with elongated thin processes was found underneath the endothelium and was very thin. In deeper regions (second and third *en face* samples), the arrangement of cells in the network became more compact. The number and length of cellular processes decreased toward the intima–media border and the cells appeared to be more elongated and spindle shaped (Figure 2D). Apart from fusiform smooth muscle cells, the subendothelial intima occasionally had round or ovoid mononuclear cells, previously identified as lymphocytes.[17] These cells had a round body 6 to 7 μ in diameter, an optically dense round nucleus, that filled the whole cytoplasm. Most frequently, ovoid cells were detected in the first *en face* preparation (adjacent to the endothelium); however sometimes they were seen in deeper regions of the elastic-hyperplastic layer.

Scanning electron microscopy was used to study the fine structure of cellular interconnections and the cell surface microrelief of grossly normal intima. Treatment with dispase and maceration in HCl and collagenase showed the cytoarchitecture of the subendothelial surface intimal layer. In these preparations, the remains of endothelial cells were sometimes identified. In preparations of the uppermost layer, a network consisting of predominantly stellate-shaped cells was found (Figure 3A). The surface of most cells had a smooth appearance. In some areas,

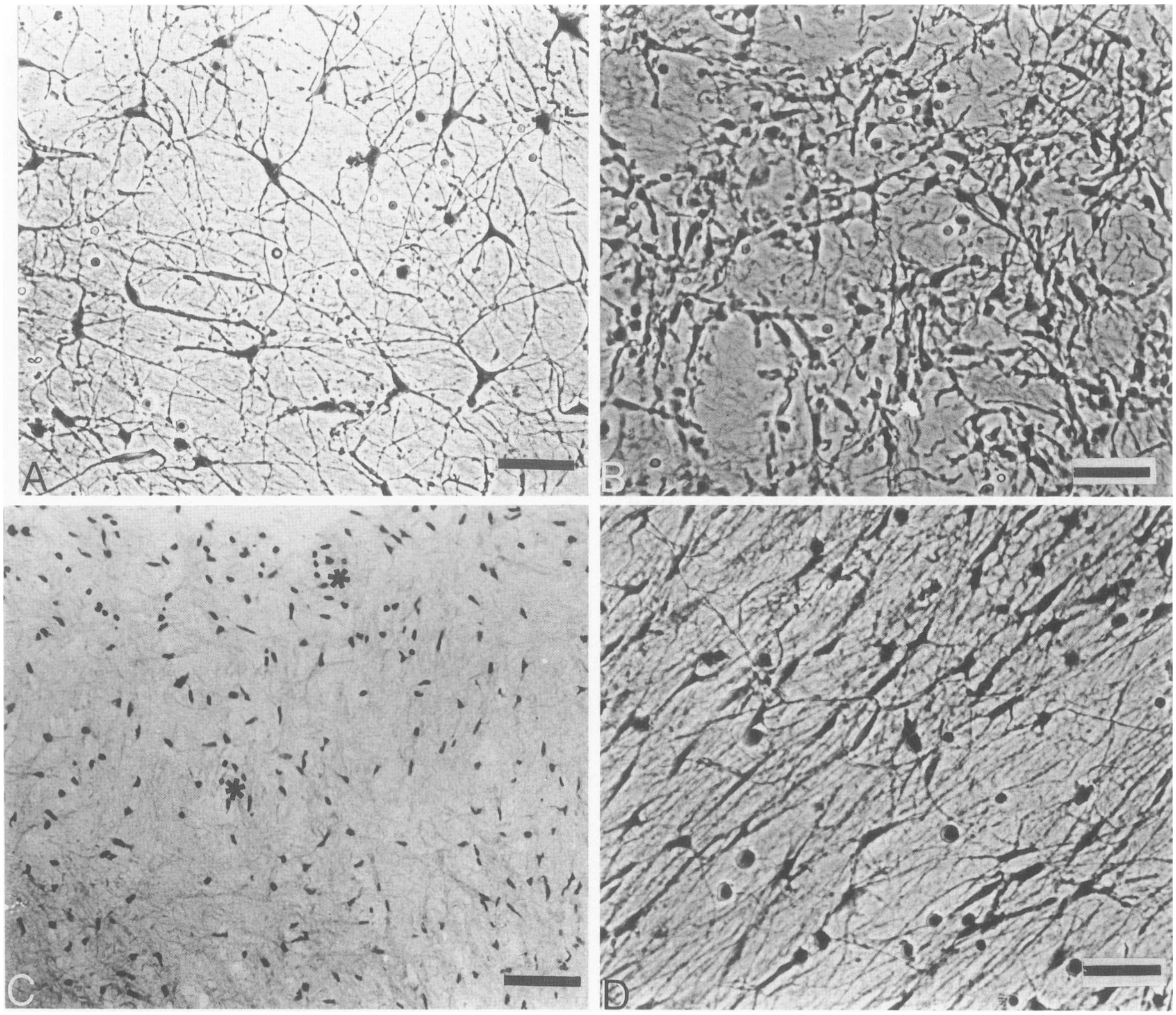

Figure 2. *Grossly normal region of the human aortic intima (light microscopy).* **A:** *Surface region of the elastic-hyperplastic layer. A two-dimensional network consisting of predominantly stellate cells. Hematoxylin, phase-contrast microscopy (bar, 50 μ; ×200).* **B** *Same as in* **A.** *Cell groups are interposed by sparse regions. Hematoxylin, phase-contrast microscopy (bar, 50 μ; ×200).* **C:** *Cell clusters are also found on horizontal paraffin section. Asterisks indicate clusters of subendothelial cell nuclei. Hematoxylin (bar, 125 μ; ×80).* **D:** *A deeper area of the elastic-hyperplastic layer. Most of the cells are spindle shaped. Hematoxylin, phase-contrast microscopy (bar, 50 μ; ×200).*

undigested extracellular matrix components were seen at the cell surface. As visualized by SEM, subendothelial cells formed contacts with each other in one plane or with cells located at different levels (Figure 3B). We failed to identify a single cellular process that was not connected with some other cellular structure. The spaces between cellular processes reaching down into the intima were filled by processes coming up from cells beneath. Smooth muscle cells in the network formed contacts of two types, between the processes of different cells and between the process of one cell and the body of another.

The nature of smooth muscle cell arrangement in the musculoelastic layer differed fundamentally from that of the elastic–hyperplastic layer (uppermost region). In the musculoelastic layer, cells were closely packed, forming a common cellular layer with small intercellular spaces (Figure 3E). Smooth muscle cells of this layer were predominantly elongated; some of them had short side processes that formed contacts with neighboring cells. Few cells of the musculoelastic layer deviated from with the regular organization and stretched across the main layer or were arranged at an angle to it.

Thus we have identified a three-dimensional network in the human aortic intima composed of cells of various shape. The shape of these cells varies from predominantly stellate in the uppermost regions of the elastic-hyperplastic layer to mostly spindlelike cells in the musculoelastic layer. We did not detect any difference in the three-dimensional structure of the musculoelastic layer of normal and atherosclerotic intima. Therefore, we will further present data on atherosclerotic changes that occur in the cellular network, only in the hyperplastic layer.

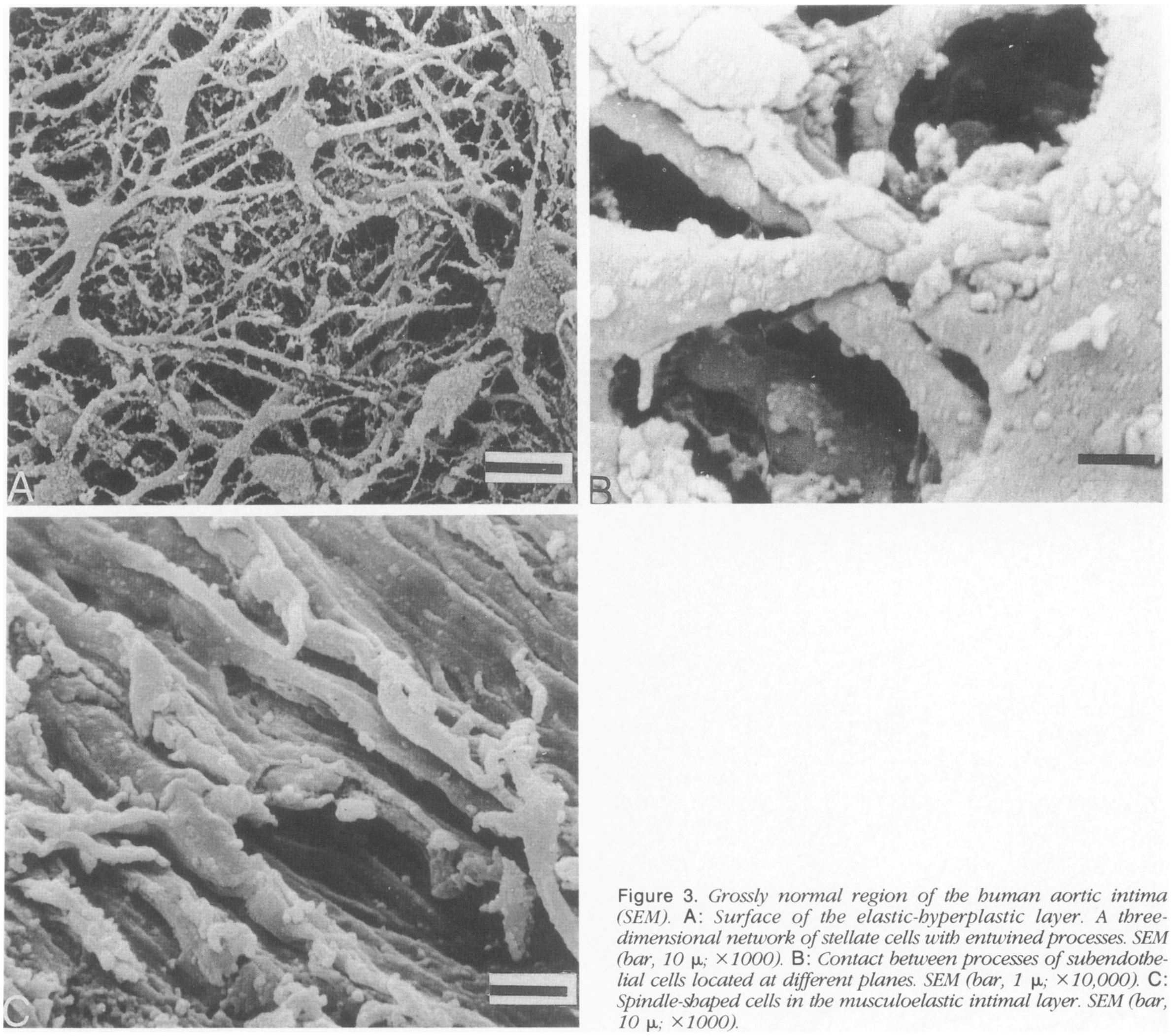

Figure 3. *Grossly normal region of the human aortic intima (SEM).* A: *Surface of the elastic-hyperplastic layer. A three-dimensional network of stellate cells with entwined processes. SEM (bar, 10 μ; ×1000).* B: *Contact between processes of subendothelial cells located at different planes. SEM (bar, 1 μ; ×10,000).* C: *Spindle-shaped cells in the musculoelastic intimal layer. SEM (bar, 10 μ; ×1000).*

Fatty Streak

From a single sample of the subendothelial intima of a fatty streak we were able to obtain at least three to four *en face* preparations. Three main cellular types could be distinguished in the surface regions of the fatty streak. First there were stellate intimal cells without lipid inclusions (similar to the majority of cells in the unaffected intima) and intimal cells with processes and intracellular lipids (Figure 4A). These cells were located in the surface layers of the fatty streak and were much larger than the stellate cells without lipids. Second round foam cells similar to macrophages described in atherosclerotic lesions by other researchers[17,18] were also found in the uppermost intimal layer. They were ovoid and had a lipid-laden cytoplasm (Figure 4B). Furthermore *en face* preparations of fatty streaks contained cells resembling blood lymphocytes.

Various *en face* preparations showed that in the surface regions of the fatty streaks intimal cells are arranged into thin microbands, which like macrobands are oriented parallel to the long axis of the vessel (Figure 4C). Four types of microbands organized by the three major cell types were found: 1) bands formed by lipid-laden intimal foam cells with elongated processes; 2) bands formed by cells with processes, but lacking lipid inclusions; 3) bands formed predominantly by ovoid foam cells resembling monocytes–macrophages; 4) bands of mixed type formed by round foam cells and foam cells with processes (Figure 3D). In the surface layer of the fatty streak, the cells were arranged with lesser regularity than in the unaffected intima. Large clusters of cells alternated with broad 'empty' spaces filled by connective tissue matrix components. Occasionally, separate cells without intercellular contacts were found.

Cellular composition changed dramatically in the deeper intimal layers of the fatty streak. At the levels of the third and fourth *en face* preparations (approximately in

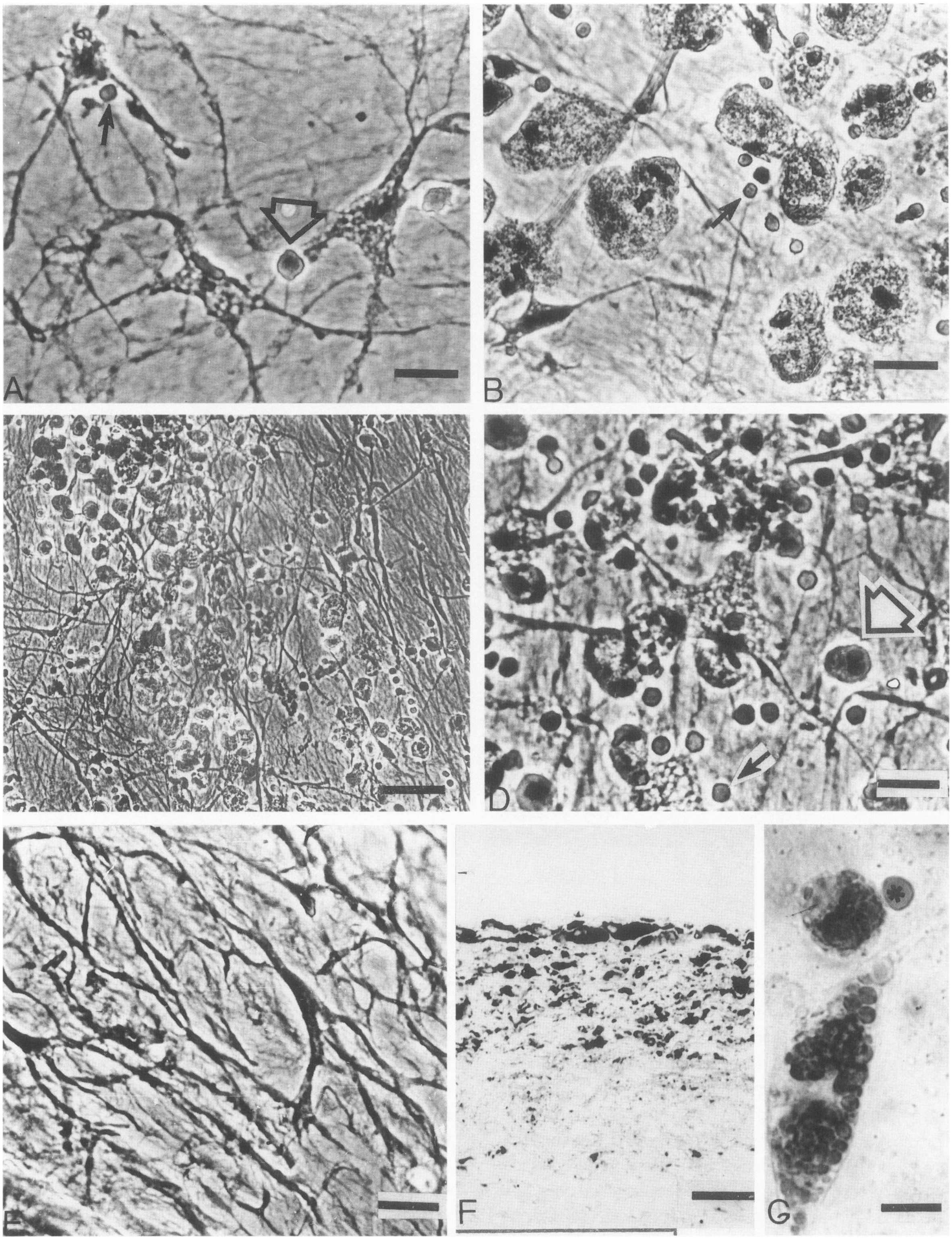

Figure 4. *Fatty streak (light microscopy).* **A–E** En face *preparations. Hematoxylin, phase-contrast microscopy.* **A**: *Surface region of the juxtaluminal layer. Accumulation of foam stellate cells (bar, 20 μ; ×500).* **B**: *Same as in* **A**. *Accumulation of round and ovoid foam cells (bar, 25 μ; ×400).* **C**: *Same as in* **a**. *"Microband" of foam cells alternating with "microbands" of cells without inclusions (bar, 125 μ; ×80).* **D**: *Accumulation of foam cells of variable shapes (bar, 20 μ; ×500).* **E**: *A deeper area of the juxtaluminal layer. Intracellular lipid inclusions are practically absent (bar, 25 μ; ×400).* **F**: *Cross section. Lipids accumulate predominantly in the juxtaluminal intimal layer. Oil red 0 (bar, 83.3 μ; ×120).* **G**: *Total* en face *preparation of juxtaluminal intimal layer. Intracellular and extracellular accumulation of neutral lipids. Arrow indicates extracellular lipid droplet. Oil red 0 (bar, 20 μm; ×500). Arrows indicate red blood cells, arrowheads indicate lymphocytes elsewhere on A, B, and D.*

the middle of the elastic–hyperplastic layer), a cellular network resembling that of the surface layer of the unaffected intima was detected (Figure 4E). The further away from the vessel lumen, the less often could intimal cells with lipid inclusions be seen. Distinct monocytelike cells were seldom found in the second from the lumen layer as compared with the first (no more than five cells per field). In the third *en face* preparation, monocytelike foam cells were absent, and stellate cells without lipid inclusions became abundant. Cross sections as well as total *en face* preparations also showed that intracellular and extracellular neutral lipids were distributed mainly in the upper regions of the hyperplastic intimal layer (Figure 3F, G).

Scanning electron microscope examination of the uppermost intimal regions of fatty streaks confirmed the cellular heterogeneity of the network (Figure 5A). Cells had an enlarged cellular body and numerous blebs formed by intracellular lipid droplets (Figure 5B). Many round and ovoid structures were found in close proximity to the intimal cell blebs and extensions (Figure 5C, D). Their dimensions were variable, with a mean of 4.6 $\pm$ 0.3 μ. These structures had rather smooth surface, but we can not exclude completely that the surface of this structures was modified under the influence of hydrolysis. Ultrathin sections showed that the extracellular matrix contained membrane-coated lipid droplets of the same size and shape (Figure 5E). Thus it seems probable that the structures visualized by SEM are membrane-coated lipid droplets. Sometimes such structures were found in close contact with cellular surfaces, suggesting that they were blebs detaching from the cell's surface. Sometimes blind cellular extensions, ie, those lacking contact with neighboring cells, were seen (Figure 5F). Apparently, extracellular lipid membrane-bound droplets are the product of foam cell exocytosis or the result of foam cell death.

Thus comparing the cytoarchitecture of the surface layers of grossly normal intima with fatty streaks, several distinctive features can be noted. First in the fatty streak intima, contacts between subendothelial cells appear to be severed. Second the uppermost layers of the fatty streak along with stellate and spindle-shaped cells contain round mononuclear cells identified earlier by electron microscopy as monocytemacrophages. Finally the subendothelium has the largest number of foam cells. Lipids accumulate primarily in round and stellate cells. Lipid-laden cells are arranged unevenly throughout the horizontal plane of the streak, forming 'microbands' of variable cellular composition.

Atherosclerotic Plaque

Four to six successive *en face* preparations of the subendothelial intima of a plaque could be obtained, depending on its size and thickness. The nature of the cellular network of the surface layers was determined by the type of the atherosclerotic plaque. The surface layers of fibrous plaques (first and second *en face* preparations) consisted mostly of crude fibrillar extracellular matrix. Separate intimal cells and groups of cells unrelated to the common network were also found (Figure 6A). In the matrix feltwork, we often saw small cells with a round nucleus and a thin rim of cytoplasm, resembling lymphocytes, and larger cells with a beanlike nucleus similar to cells of the mononuclear linage. In deeper layers of the plaque fibrous cap, the number of cells increased; however they did not form a common network. Giant flat cells (Figure 6B, C), singularly distributed or in groups, were seen.

The surface part of the plaque necrotic core consisted of giant cells and their fragments, rarely distinguishable from the matrix and degenerating blood cells. In deeper core regions, necrosis was more pronounced, and cell debris, deposits of extracellular structures and lipids, remains of blood cells, and extracellular matrix components were found (data not shown).

The organization of the lipid–fibrous plaque cap differed from that of the fibrous plaque cap. The upper layer consisted of a combination of pieces of extracellular matrix, necrotic mononuclear cells, and singular or associated in groups lipid-laden stellate intimal cells (Figure 6D). Deeper layers contained a well-developed extracellular matrix network with sparsely distributed intimal cells, extracellular lipids, and many mononuclear cells with a diameter of about 10 μ. Sometimes regions with stellate and ovoid foam cells were found. As in fatty streaks, these cells formed microbands. The third and fourth *en face* preparations obtained from the plaque cap disclosed the presence of giant cells arranged over the atheromatous core in a manner similar to the structure of the fibrous plaque core.

At the slopes of plaques (irrespective of plaque type) in the surface layer of the intima, we found a sharp border between the cap and the marginal zone of the plaque. In the cap, the cells were arranged loosely, whereas in the surface intimal regions adjacent to the plaque, cells formed a dense and elaborate framework (Figure 7).

In the surface layers of fibrous plaque cap, SEM showed a massive extracellular matrix that consisted of thick fibers. These fibers were composed of the connective tissue fibrils, which seemed to be more crude than in unaffected regions. In lipid–fibrous plaques, the extracellular matrix fibrils were thinner and irregularly distributed compared with the fibrils of fibrous plaques. In deep regions of fibrous plaques, giant flat cells entwined by densely packed connective tissue fibrils were often seen (Figure 8A). In deep regions of lipid–fibrous plaques, stellate cells with blebs and extracellular ball-like structures with slightly indented surface were seen (Figure 8B).

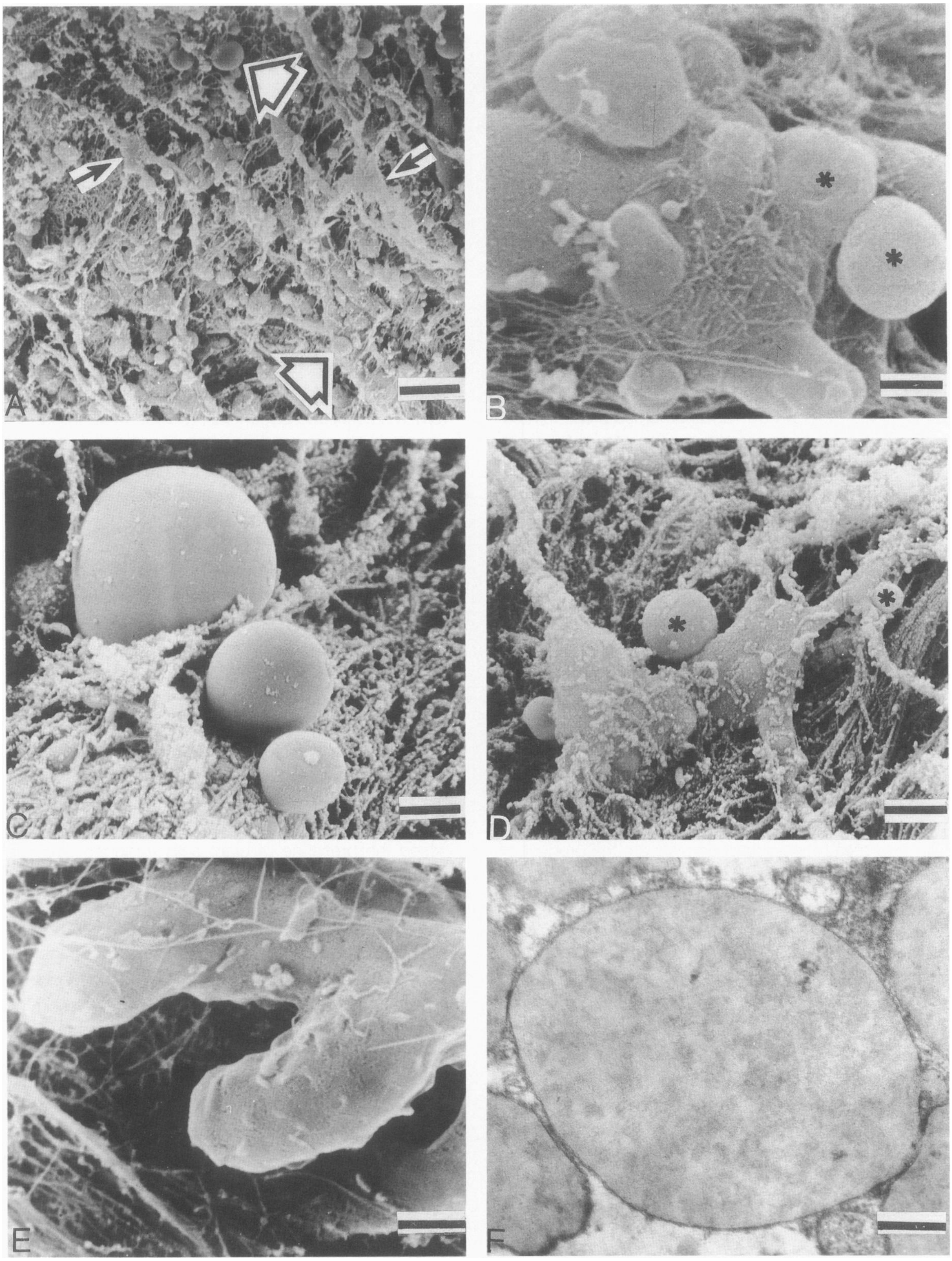

Figure 5. *Fatty streak (electron microscopy).* **A**: *The network of subendothelial cells in the uppermost intimal region. Arrows indicate stellate subendothelial cells, arrowheads indicate extracellular ball-like structures. SEM (bar, 25 μ; ×400).* **B**: *Subendothelial cell with a large cellular body and bleblike protrusions (indicated by asterisks). SEM (bar, 1 μ; ×10,000).* **C**: *Extracellular ball-shaped structures (indicated by asterisks) are situated just near the body and processes of the cell. SEM (bar, 2.2 μ, ×4500).* **D**: *Accumulation of extracellular ball-like structures of various dimensions. SEM (bar, 3.3 μ; ×3000).* **E**: *Ultrathin section of the re-embedded sample. Membrane-coated lipid droplets are seen. TEM (bar, 333 nm; ×30,000).* **F**: *Impaired contacts between subendothelial cells. SEM (bar, 660 nm; ×15,000).*

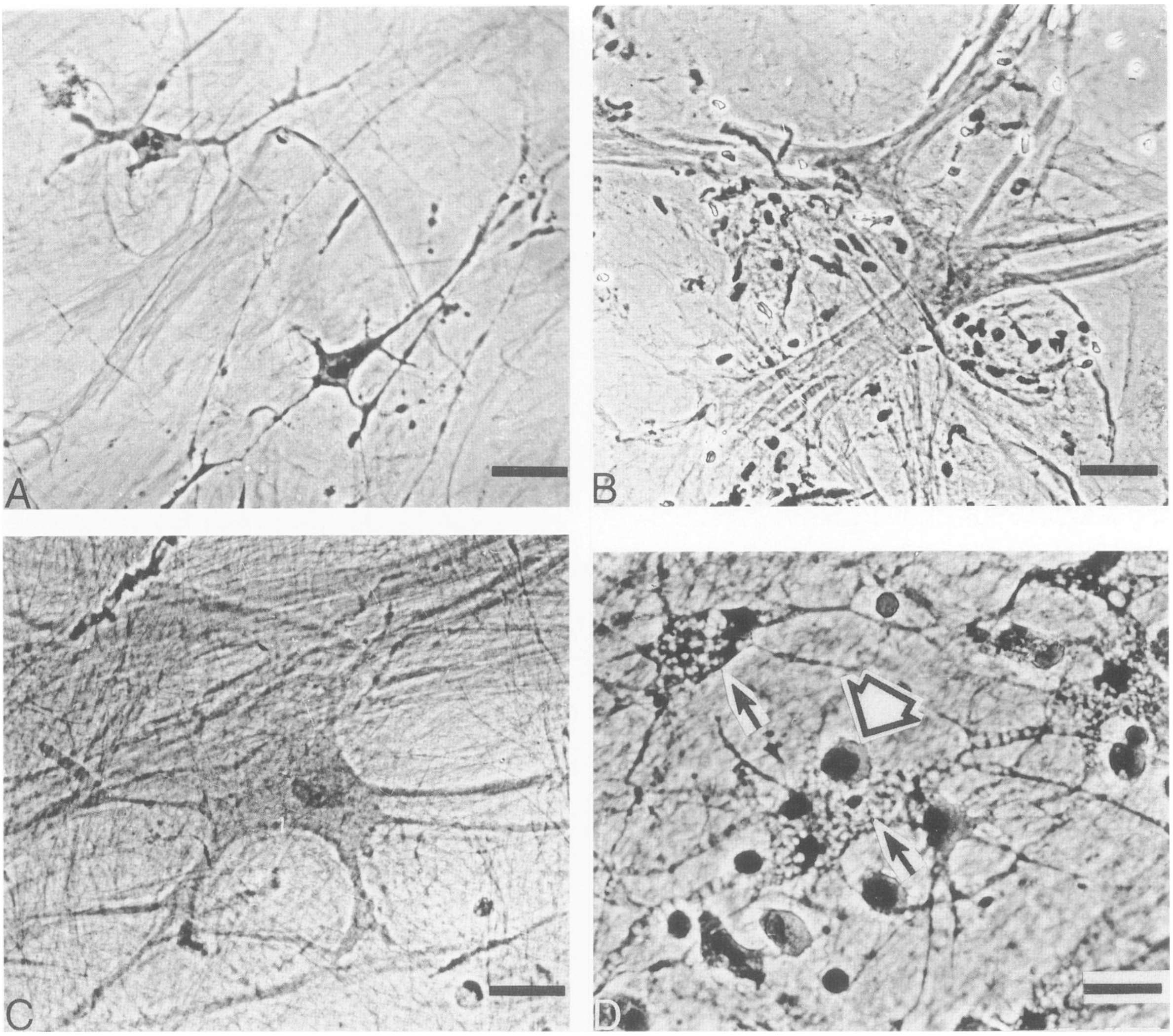

Figure 6. *Atherosclerotic plaque.* En face *preparations. Hematoxylin, phase-contrast microscopy.* **A**: *Surface region of fibrous plaque cap. Dissociated cells with elongated branching extensions (bar, 50 μ; ×200).* **B**: *Deeper region of the fibrous plaque cap. Two giant cells laying in close contact are seen (bar, 50 μ; ×200).* **c** *same as in* **b**. *A single stellate giant cell (bar, 50 μ; ×500).* **D**: *Lipid-fibrous cap. The arrows indicate stellate foam cells. Contacts between them are imperfect. The arrowhead indicates lymphocytes (bar, 25 μ; ×400).*

The atheromatous core contained fibrillar matrix embedded with homogenous laminae resembling the remains of intimal cells and degenerating blood cells. We found extracellular lipid droplets in the atheromatous plaque core similar to those present in fatty streaks described earlier. Apart from fatty streaks, however, which had many round-shaped lipid droplets only in the surface intimal layer, these lipid structures were irregular in shape, elongated, and had large and deep indents in the surface (data not shown). These structures were detected only in deep layers of atheromatous core.

Scanning electron microscopic examination of intima in areas adjacent to the plaque base showed that the cellular architecture is essentially the same as in grossly normal regions of intima. Cells of regular dimensions formed a network that interconnected each cell in the horizontal and vertical planes.

Thus the distinctive characteristic of the three-dimensional cytoarchitecture of the atherosclerotic plaque cap is the disruption of the intimal cellular network integrity. The cellular network 'breaks off' at the slope of the plaque. The main cell population consists of giant stellate flat cells embedded in a crude fibrous connective tissue matrix.

Discussion

We have previously used mechanical dissection of the aortic wall into separate layers for morphologic and biochemical purposes.[4,7] The tissue was divided along the elastic membranes, which served as natural borders between the intima and the media, as well as between the musculoelastic and elastic–hyperplastic sublayers of the intima. In the present study, we show that the elastic–

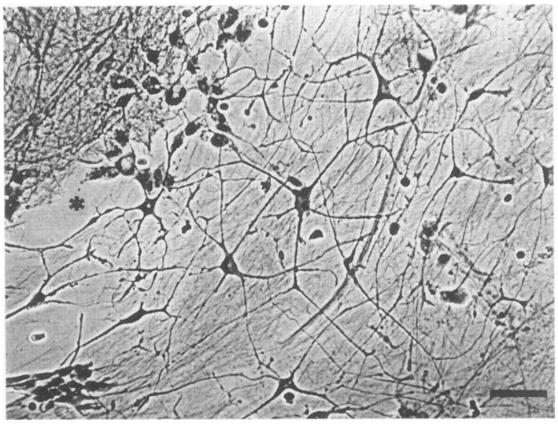

Figure 7. *Base of fibrous plaque. A network of stellate cells that 'break' at the slope of the plaque (indicated by the asterisk) (bar, 50 μ; ×200).*

hyperplastic layer can be divided even further using the technique of *en face* tissue preparations. Evidently, this is due to the fact that the cells are arranged in the subendothelium in a regular manner, as has been demonstrated by Tracy et al,[19] and are separated by reticuline fibrils into layers. The examination of cell layer structures on *en face* celloidin preparations combined with SEM was the methodologic basis of this work.

In our opinion, specific interest in the cytoarchitecture of the normal subendothelial intima has presented the following findings:

the three-dimensional cellular network in the intima
the cluster nature of the cellular network
the variations in cellular shape evident from the media toward the endothelium.

Usually conventional cross sections of the aortic wall show separate round or spindlelike cells.[5,6,18,19] Of primary interest, therefore, is the presence of stellate cells found in *en face* preparations of the subendothelial intima. We have also studied the cellular polymorphism of the human aortic intima after cells were isolated by alcohol–alkali treatment.[4,7] This method does not, however, show the full picture of cellular arrangement in the vessel wall. Schonfelder,[3] who studied horizontal sections of the intima, came to the conclusion that the cells are joined into a common network. He found empty spaces between interlaced cellular extensions, which, according to his proposal, were channels reaching deep into the intima. Our results, essentially those obtained by SEM, imply that in the human aortic unaffected intima all cells are interconnected in the horizontal plane and form a common network. The cells are also joined to each other in the vertical plane, thus developing contacts between networks of different levels.

We failed to find a single cellular process that did not form contacts with the body or process of another cell. Therefore the subendothelial intima can be regarded as a common cellular system arranged into a network, rather than a sum of isolated cells. We also detected spaces between cellular extensions filled with extracellular matrix components, which might be suggested to be responsible for the intratissue metabolism of the intima.

Of substantial importance is the cluster nature of the cellular network, which has loose and condensed regions similar to the 'hills and valleys' characteristic of cultured smooth muscle cells.[20] In the three-dimensional framework, the knot of the cellular net becomes the analog of the 'hill' where cells form the greatest number of contacts

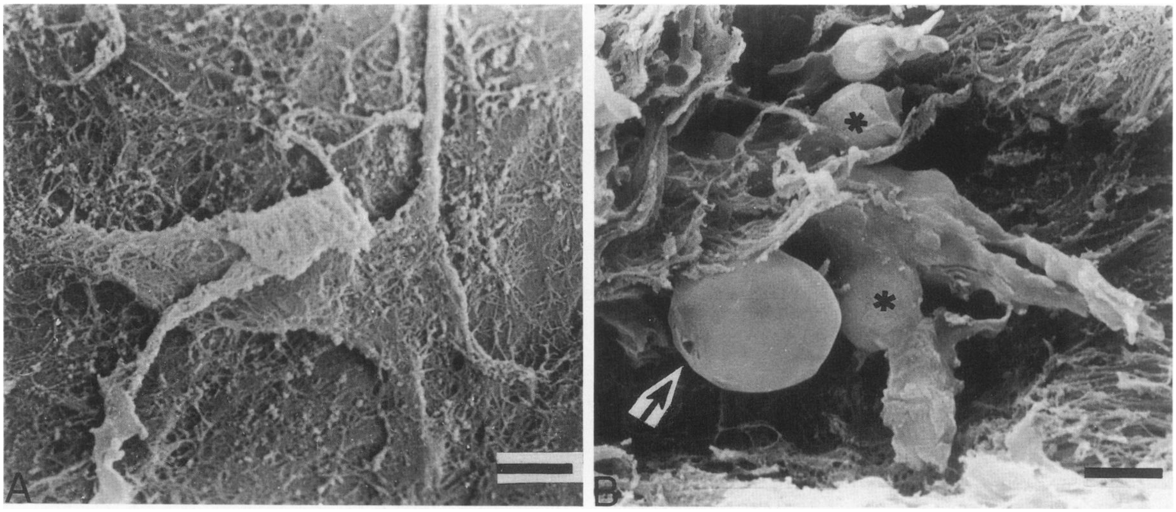

Figure 8. *Atherosclerotic plaque (SEM).* A: *A deeper region of the fibrous plaque cap. Giant flattened cell, 'encapsulated' in a fibrillar connective tissue matrix (bar, 10 μ; ×1000).* B: *a deeper region of the lipid-ibrous plaque. Stellate cell with blebs (asterisk). Arrow indicates extracellular ball-like structure with slightly indented surface (bar, 2.9 μ; ×3500).*

(maximum number of contacts formed by the central cell) between each other than with cells of other knots. It cannot be ruled out that such a knot of cells is a clone formed by a single cell in the center. Conversely, it can be suggested that the appearance of clusters in the cellular network represents one of the earliest features of pathologic changes in the subendothelial intima that lead to the complete degradation of the network as seen in atherosclerotic lesions.

Schonfelder[3] noted another important fact, ie the increase in the number of branched cells from the internal elastic membrane to the endothelium. We also found similar alterations in cell shape, taking into consideration that there is no strict anatomic border between the areas populated by stellate and spindle-shaped cells. In unaffected intimal regions, the change in cell shape takes place gradually without disturbing the integrity of the network. The origin of stellate cells remains unclear[21]; however our data on the gradiential structure of the cellular network support the suggestion that stellate cells are the progeny of spindle-shaped cells, formed under the influence of some factors permeating from the vessel lumen or from the endothelium.

What is the nature of alterations that occur in the cellular network during atherosclerosis? In our opinion, the most important new information presented in this study concerns the degradation of the cellular network in atherosclerotic lesions (the destruction of intercellular contacts in the fatty streak and the total dissociation of intimal cells in the atherosclerotic plaque cap). The mechanism whereby intimal cells lose contact with each other is unknown. We suggest that one of the causes of the destruction of the integrity of the cellular network could be the accumulation of intracellular lipids. Usually intimal cells are heavily laden with lipids in fatty streak, which obviously leads to increased cell dimensions and the formation of surface bleblike protrusions by lipid droplets and vesicles. Simultaneously, round structures identified on cross sections as membrane-coated lipid droplets appear near the cells. Similar structures have been recently described by Guyton and Klemp.[22] It seems likely that these lipid structures are propagated by gemmation from the surface of the cell; however additional ultrastructural investigation is needed to substantiate this proposal. Vesicle gemmation from the ends of cellular extensions can obviously lead to the degradation of intercellular contacts and to cell dissociation.

Therefore specific interest relates to the question of which cells predominantly accumulate lipid and where are they located in the cytoarchitecture of the intima. Cellular composition of fatty and fibrous lesions has gained much attention.[8,11] Most studies, however, were performed on cross sections, which offer little information on the shape and contacts of intimal cells. The present investigation provides a detailed description of lipid-laden cells arranged predominantly in the uppermost regions of the fatty streak. Lipids accumulate primarily in stellate intimal cells and in monocytelike ovoid cells. Foam cells form groups in the horizontal plane, which become microbands. The microband, by its degree of the cellular network disintegration and the number of stellate intimal cells, resembles atherosclerotic plaque. Furthermore it cannot be excluded that these microbands could be the direct precursors of plaques. Insofar, however, it is unclear whether a link between the destruction of cellular network integrity in the fatty streak and the development of atherosclerotic plaques exists. It remains a fact that the process of cellular network destruction that begins in the fatty streak terminates in the fibrous plaque cap with the total dissociation of cells from each other and from the cellular network in the bordering slope areas of the plaque.

In our view, the extreme form of cellular isolation is the 'embedding' of intimal cells in crude fibrils of connective tissue material. Previously cells surrounded by a thick connective tissue capsule have been described in atherosclerotic plaques.[6] The formation of connective tissue capsules, apparently, isolates cells from each other, as well as significantly impedes their metabolism, resulting in intimal dystrophy.

Thus because of new methodologic procedures we were able to demonstrate the presence of a three-dimensional cellular network in the human aortic intima, the integrity of which is severely disrupted in atherosclerotic lesions.

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